

## STUDIES ON THE KARYOTYPE OF THE RED-TAILED HAWK (*BUTEO JAMAICENSIS*)

by

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### *Abstract*

Feather pulp from growing body feathers of Red-tailed Hawks (*Buteo jamaicensis*) located at the Raptor Center at the University of California, Davis, was treated in a hypotonic solution and fixative, then squashed and stained with Giemsa. The resulting spreads showed a majority of 34 pairs ( $2n=68$ ). The sex chromosomes were the fourth pair of the series; the female had heterogametic and the male homogametic pairs. The Common buzzard (*Buteo buteo*), reported by an earlier investigator, had a diploid number of 68 with the sex chromosome pair number as fourth in the series. Analytical methods showed that subjective judgment of the relative size of the chromosomes is fairly accurate, though ideally it would be best to analyze before the karyotypes are permanently mounted.

### *Introduction*

Birds of prey are not always sexually dimorphic. Ease of selection for breeding pairs in captivity has been handicapped for some less dimorphic species. The usual method of sexing raptors is by weight; yet many birds have weights that fall in an overlap range between males and females. This project was undertaken to provide essential information for the breeding project at the University of California, Davis's Department of Avian Sciences' Raptor Center, and to obtain the karyotype of a common North American raptor, the Red-tailed Hawk (*Buteo jamaicensis*).

Another criterion for this project was to not physically and permanently impair the birds though most will never be free again since they have been crippled prior to coming to the Raptor Center. Thus, a method that produced a high mitotic index yet did not injure the birds during the procedure was used (Sandes 1954, Panchenko 1970). It entailed injecting a nonpoisonous mitotic arrester (Colcemid, demecolcine) which prevents the formation of the mitotic apparatus required for normal cell division (Brinkley et al. 1967). This procedure allowed for collecting cells that had their chromosomes distinctly formed and separated, easily counted, and then laid out in a distinctive arrangement known as a karyotype.

### *Materials and Methods*

The two birds used in this study were residents in the Avian Sciences' Raptor Center and were either mature adults or just recently matured birds. The dosage was 1 ml of 0.05% Colcemid per 680 gms bodyweight (Jensen 1967). The calculated amount was injected intravenously using the brachial vein crossing over the phalangeal joint. After 50 minutes, growing body feathers were pulled, the pulp was removed from the base using forceps, and it was put into a preheated 0.45% trisodium citrate solution with 0.001% colchicine final concentration added for additional mitotic arresting. The tissue was left in this hypotonic solution (at 40°C) from 20 to 30 minutes, depending on the age of the bird (a longer time for older birds). The feather pulp then was re-

moved with forceps from the hypotonic solution and placed into freshly made fixative (50% acetic acid). The tissue was allowed to remain in the fixative at last 30 minutes and no longer than one hour. The tissue was placed on a cleaned slide and macerated with a scalpel. Each slide and coverslip was cleaned with Comet prior to use and rinsed with 2X distilled water. After a sufficient suspension was produced, a drop of the solution was rolled onto another precleaned slide and covered with a coverslip, and the tissue was squashed.

For uniformity in the squash technique, a "squash box" was constructed. It was made of plastic with a square section in the center where the slide was secured. A plastic lid fit over the slide, and a clamp was used to apply pressure to the lid that was transferred to the slide under the lid. This method dispersed the cells into a single layer. After squashing, each slide was put into a dry ice-100% ethanol (EtOH) bath that had been sitting for 30 minutes. The slides were left in for a few seconds so that the coverslips could be flicked off with a razor blade leaving the tissue intact on the slide. The slides were then transferred into 100% EtOH for 5 minutes, and then into preheated 1N HCl (56–58°C) for 6 minutes. It was imperative that the temperature be controlled, or the spreads would not stain properly. The slides were then rinsed in running tap water for 5 minutes, and finally rinsed in distilled water. They were stained with normal Giemsa for 15 to 20 minutes, rinsed, and air dried. Clean coverslips were mounted with piccolyte.

### *Observations and Discussion*

One of the most easily seen characteristics of chromosomes is their attachment during mitosis at their centromeres. Many researchers have described chromosomes using the position of the centromere as a central focal point. To bring a consistency in comparison of the various reports on chromosomes, the Denver Study Group devised a mathematical formula for determining the location of the centromere of the chromosomes (Levan et al. 1965).

The analytic procedure consisted of measuring the long and short arms of the chromosomes and averaging the pair, then using the formula:  $100s/l+s$ , where  $s$  = the short arm of the chromosome and  $l$  = the long arm of the chromosome. Levan et al. (1965) formulated a table relating the results that made it possible to find the centromere locations of the chromosomes of these two karyotypes (Table 1).

Both sexes of *Buteo jamaicensis* had variable numbers of chromosomes, but the number which occurred most frequently was 34 pairs ( $2n = 68$ ). Accordingly, the observations and discussion are based on this number.

The results of the Red-tailed Hawks for the first thirty pairs are shown on Table 2. As could be seen in Table 2, all but eight pairs did not exactly correspond, though only four were not close in their location of the centromere.

The Red-tailed Hawk karyotype appeared to have mainly terminal point (T) (41.2–44.0%) and median region (m) (35.3–38.2%) chromosomes. The submedian (sm) constituted the third largest group (20.6–23.5%) with median point (M) and sub-terminal (st) chromosomes each making up 2.9%. The most interesting aspect of this karyotype was the small number of microchromosomes of which most other birds, notably the domesticated species, have such a large number that it is difficult to count. In descending order of size, pair number four were believed to be sex chromosomes. The male had a homogametic pair (figure 2); the female had a heterogametic pair (figure 4).

Renzoni and Vegni-Talluri (1966) examined karyotypes of several raptorial species,

Table 1. Centromere location and centromeric index (i)

Nomenclature	i
M	50.0
m	47.5 45.0 42.5 40.0 37.5
sm	35.0 32.5 30.0 27.5 25.0
st	22.5 20.0 17.5 15.0 12.5
t	10.0 7.5 5.0 2.5
T	0.0

The terms used herein are:

Term	Location	<i>i</i> value
M	median point	50.0
m	median region	47.5-37.5
sm	submedian region	37.5-25.0
st	subterminal region	12.5-25.0
t	terminal region	2.5-12.5
T	terminal point	0.0

including the Common Buzzard (*Buteo buteo*). The diploid number of chromosomes in the Common Buzzard was found to be 68; the sex chromosomes were the fourth pair. These findings were very similar to those on the Red-tailed Hawk data in this investigation.

### Conclusion

In the Red-tailed Hawk, chromosome numbers ranged from 32 to 36 pairs, but the majority had 34 pairs ( $2n = 68$ ). This is an adequate estimate of the chromosomal number for this species though it is by no means unequivocal.

The most common sex-determining method used currently involves the use of weight since the female is usually the heavier. However, other methods are becoming available. Dr. Arthur Risser, Jr., is examining the excrement of birds for steroid levels (Risser 1977), a method which may prove successful but requires use of radioactive

Table 2. Data on the female Red-tailed Hawk chromosomes

	l	s	i	term
	1.35	0.9	40.0	m
	1.4	0.7	33.3	sm
	1.2	0.7	36.8	sm
(Z)	1.25	0.55	30.6	sm
(W)	0.7	0.4	36.4	sm
	1.2	0.68	36.2	sm
	0.725	0.7	49.1	m
	0.8	0.55	40.7	m
	0.95	0.25	20.8	st
	0.65	0.45	40.9	m
	0.6	0.4	40.0	m
	0.575	0.4	41.0	m
	0.55	0.4	42.1	m
	0.55	0.3	35.3	sm
	0.4	0.3	42.9	m
	0.35	0.3	46.2	m
	0.35	0.3	46.2	m
	0.4	0.2	33.3	sm
	0.3	0.2	40.0	m
	0.35	0.15	30.0	sm
	0.3	0.2	40.0	m
	0.45	0.0	0.0	T
	0.45	0.0	0.0	T
	0.45	0.0	0.0	T
	0.45	0.0	0.0	T
	0.4	0.0	0.0	T
	0.35	0.0	0.0	T
	0.35	0.0	0.0	T
	0.35	0.0	0.0	T
	0.3	0.0	0.0	T
	0.25	0.0	0.0	T

material. Cultured white blood cells could also be used, together with chromosomal spreads from them, but would require culturing media and antibiotics. The method described in this report utilized a minimum of expensive equipment and fairly easily obtainable chemicals, except for the mitotic arrester. The technique could be handled in the field though results would be better in a clean laboratory. The time involved in making the slides, from injection to permanent mounting, was relatively short, about 3 to 4 hours.

#### *Acknowledgment*

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Table 3. Data on the male Red-tailed Hawk chromosomes

	l	s	i	term
	1.45	0.75	34.1	sm
	1.15	0.8	41.0	m
	1.2	0.6	33.3	sm
(Z)	1.0	0.5	33.3	sm
	1.0	0.75	42.9	m
	0.95	0.75	44.1	—
	0.8	0.4	33.3	sm
	0.5	0.45	47.4	m
	0.5	0.45	47.4	m
	0.6	0.3	33.3	sm
	0.45	0.35	43.8	m
	0.5	0.3	37.5	sm
	0.45	0.35	43.8	m
	0.45	0.3	40.0	m
	0.4	0.3	42.9	m
	0.45	0.2	30.6	sm
	0.35	0.25	41.7	m
	0.6	0.0	0.0	T
	0.3	0.3	50.0	M
	0.3	0.25	45.5	m
	0.3	0.25	45.5	m
	0.3	0.2	40.0	m
	0.5	0.0	0.0	T
	0.5	0.0	0.0	T
	0.5	0.0	0.0	T
	0.4	0.0	0.0	T
	0.35	0.0	0.0	T
	0.25	0.0	0.0	T
	0.2	0.0	0.0	T
	0.2	0.0	0.0	T

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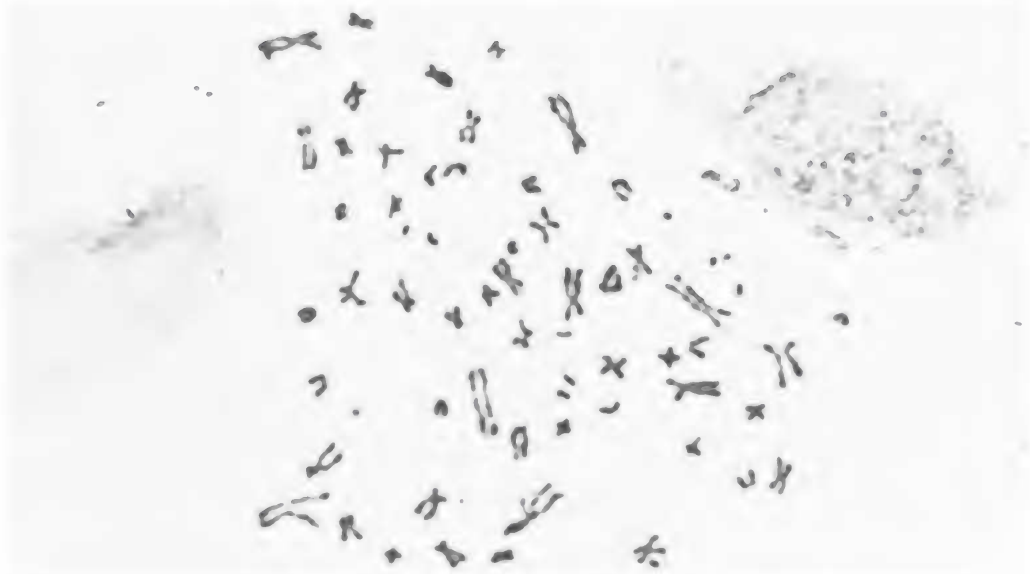


Figure 1. Chromosomal spread of male Red-tailed Hawk (*Buteo jamaicensis*) 6,550 X

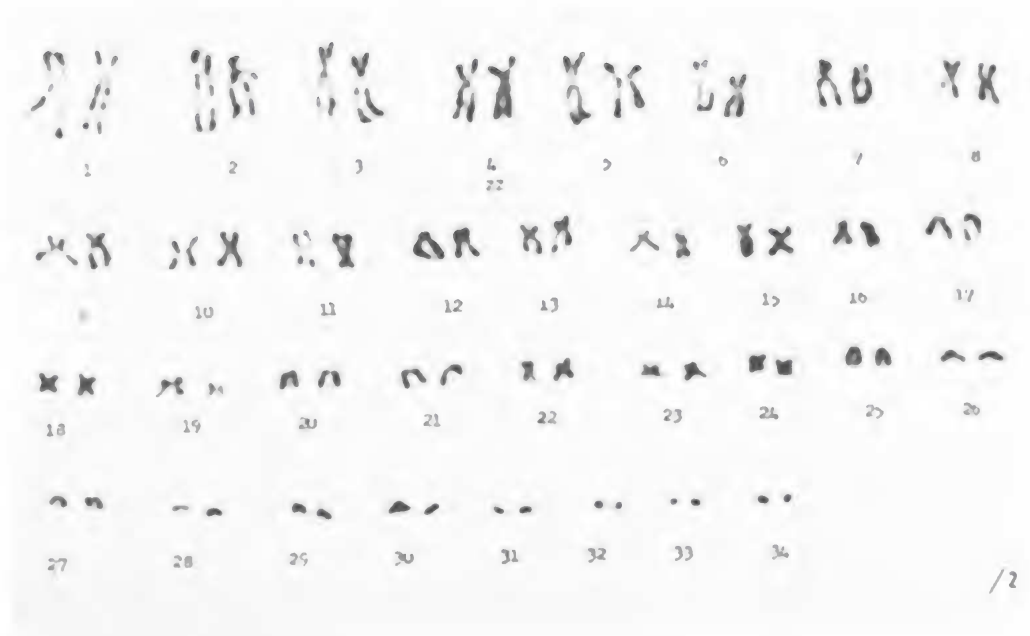


Figure 2. Karyotype of male Red-tailed Hawk (*Buteo jamaicensis*) 7,960 X

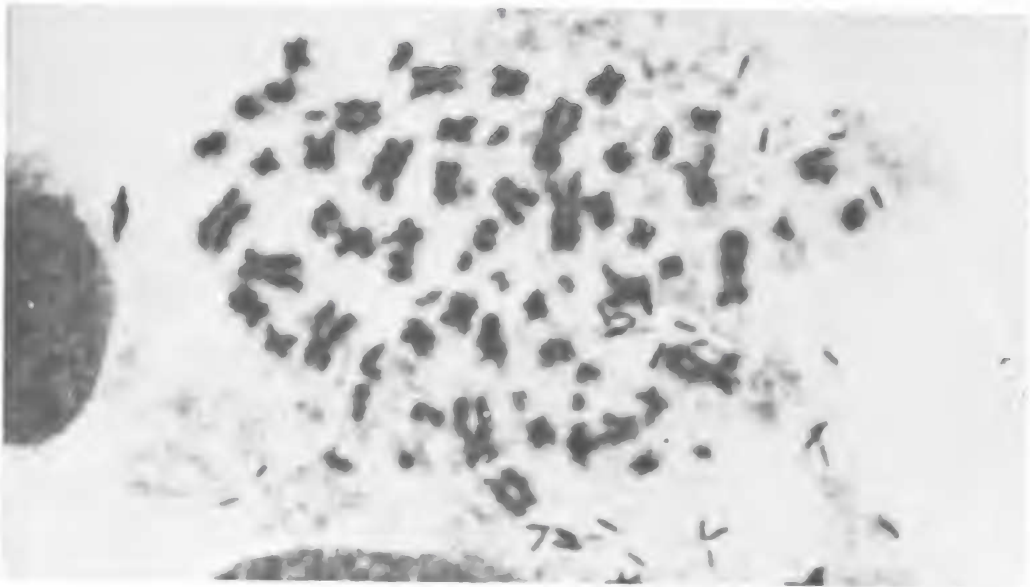


Figure 3. Chromosomal spread of female Red-tailed Hawk (*Buteo jamaicensis*) 7,670 X

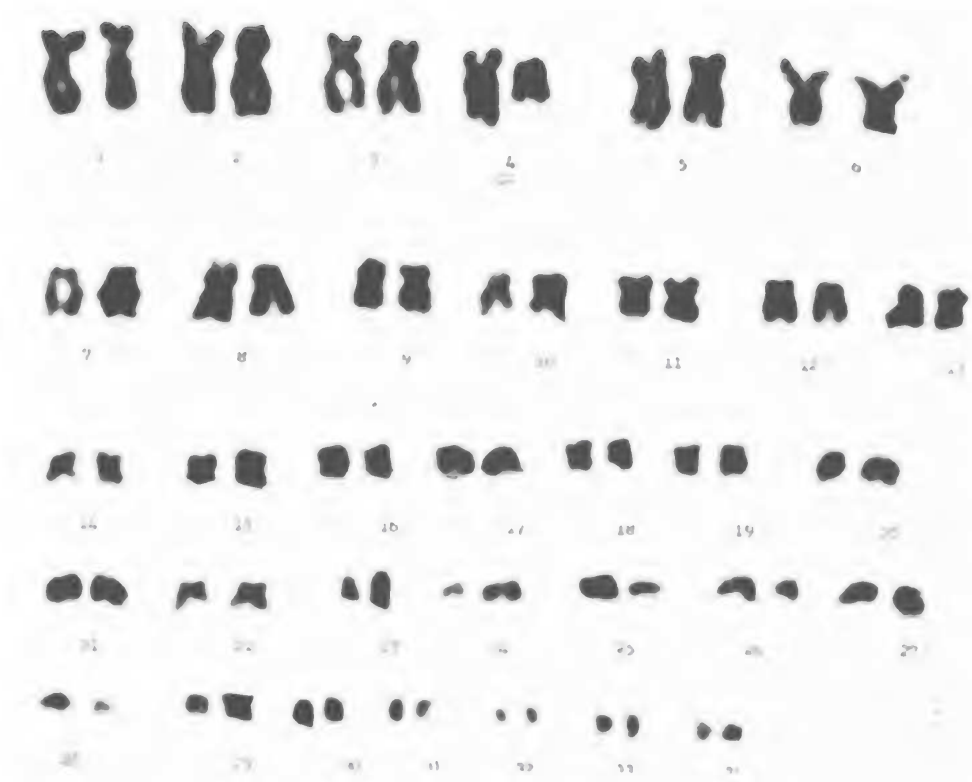


Figure 4. Karyotype of female Red-tailed Hawk (*Buteo jamaicensis*) 7,280 X